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Detection of Hepatitis C Virus Genomic Sequences in the Cerebrospinal Fluid of HIV-Infected Patients

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To assess the presence of hepatitis C virus (HCV) in the central nervous system (CNS), HCV-RNA was sought in paired serum and cerebrospinal fluid (CSF) samples of 21 HIV/HCV-positive patients: HCV-RNA was detected in the serum of 19/21 patients (90.4%), and in the CSF of five of the 19 serum-positive patients. The presence of HCV-RNA was confirmed in follow-up CSF samples available for three of these five patients. An identical HCV genotype was found in the paired serum/CSF samples. No correlation was found between the different genotypes and the presence of HCV in CSF of the individual patients.

HCV viremia levels measured by branched-DNA and quantitative PCR were not significantly higher in the CSF-positive cases than in the CSF-negative cases (P = 0.3, using b-DNA; 0.5, using quantitative PCR). This report shows the presence of HCV in CSF and raises the possibility that the CNS may act as a reservoir site for HCV. ***J. Med. Virol. 53:252–254, 1997.***

KEY WORDS: HCV; Brain; HIV

INTRODUCTION

Hepatitis C virus (HCV) is the major causative agent of blood-borne non-A, non-B hepatitis in the world and, in recent years, has been the subject of extensive nucleic acid sequence analyses [Simmonds et al., 1994] that allowed the classification of HCV within the Flaviviridae family, which is known to have tropism for neural tissue (mosquito-borne viruses, such as yellow fever and dengue virus, and the tick-borne viruses that cause tick-borne encephalitis) [Miller and Purcell, 1990]. Some reports have shown that HCV can infect peripheral blood mononuclear cells (PBMCs) [Muller et al., 1993; Chang et al., 1996]. HCV genomic sequences have also been detected in biological fluids, such as semen, urine, and saliva [Liou et al., 1992], although there is no information concerning HCV tropism for other tissues. In particular, given the genomic homology between HCV and other encephalotropic flaviviruses, it is debated whether HCV can infect the central nervous system (CNS).

The aim of this study was to analyze the presence of HCV-RNA in the cerebrospinal fluid (CSF) of patients with anti-HCV co-infected with human immunodeficiency virus (HIV), and to compare viral levels in serum and CSF. Furthermore, the HCV genotype was analyzed in paired serum and CSF samples, and a possible association between the specific genotype and HCV CNS infection was investigated.

PATIENTS AND METHODS

All patients gave informed consent to participate in virological research. The study protocol received official institutional and ethical approval; 21 patients with HIV infection were studied (16 males and 5 females; mean age 31 years), who were suspected of having an opportunistic infection or neoplasm of the CNS [Petito et al., 1986]. Computed tomography (CT) scan or magnetic resonance imaging (MRI) of the brain showed focal lesions in nine patients and diffuse lesions in six [Ramsey and Geremia, 1988]: six other patients were affected by neurological disorders without any neuroradiological abnormalities. All patients underwent a lumbar puncture for diagnostic purposes, after informed consent; the lumbar puncture was repeated in seven patients whose disease worsened (mean time interval from the first lumbar puncture; 36 days; range: 30–160 days). A total of 37 CSF samples from the 21 patients were analyzed. Serum and CSF samples were collected on the same day, and the CSF samples were subjected to physicochemical analysis, *Cryptococcus* antigen detection, and bacterial culture. The residual CSF was centrifuged at 1,200 rpm for 20 min, and the supernatant was separated from the cell-containing pellet; pellets and supernatants were stored separately at −80°C. Physicochemical CSF analysis showed a mean cell number of 30/mm^3 (range: 0–180); to exclude

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TABLE I. HCV Genotype Characterization and Viral Load in Five Patients Found Positive for HCV-RNA in CSF: Relationship to Clinical Diagnosis and CSF Biochemical Parameters*

Case No.	Clinical diagnosis	CSF analysis			Quantitative PCR[a]		Branched DNA[b]		Genotype	
		Glucose (mg/dl)	Cells (n/μL)	Protein (mg/dl)	Serum	CSF	Serum	CSF	Serum	CSF
1	Neurotoxoplasmosis	50	2	78	n.d.	n.d.	8,164	Neg	1a	1a
2	CMV encephalitis	62	5	24	3,361	4.2	37,990	Neg	1b	1b
3	Seizures, encephalitis	65	2	54	1,883	Neg	33,270	Neg	3a	3a
4	Encephalitis	39	9	350	1,452	Neg	12,910	Neg	1b	1b
5	Neurotoxoplasmosis	67	1	30	2,082	Neg	2,165	Neg	4a	4a

*HCV-RNA was confirmed positive in replicate CSF samples from patients 4 and 5, whereas intermittent positivity was observed after testing several CSF samples from patients 3 (see Results). Encephalitis of patients 3 and 4 remained of unknown origin.
[a]Viral copies × 10^3/ml.
[b]Copy equivalents × 10^3/ml.
n.d., not done; neg, negative (below cutoff, see text); CMV, cytomegalovirus; PCR, polymerase chain reaction.

CSF contamination with blood, possibly caused by the lumbar puncture, the presence of red blood cells was carefully looked for in all samples by microscopical examination of 1 μl of CSF. The mean protein and glucose content were 116 mg/dl (range: 24–350) and 47 mg/dl (range: 17–69), respectively. All patients were anti-HCV positive, 15 (71.4%) had abnormal alanine aminotransferase (ALT) levels (mean value: 107 IU/L, range: 46–365 IU/L, normal value < 40 IU/L); none had been treated previously with interferon-α (IFN-α). Total nucleic acids were extracted from the CSF supernatants and serum samples by the phenol–chloroform method, reverse transcribed into cDNA, and amplified using nested polymerase chain reaction (PCR) with primers specific for the highly conserved 5′ UTR region of the HCV genome [Morsica et al., 1995]. The specificity of the PCR products was confirmed by liquid hybridization with a ^{32}P-radiolabeled probe [Farma et al., 1995]. Anticontamination protocols, as described previously, were used throughout the procedure [Kwok and Higuchi, 1989]. To exclude the presence of HCV-infected leukocytes in the CSF, human genomic DNA in the CSF supernatants was tested by PCR for an HLA-DQ-α locus of the major histocompatibility complex [Saiki et al., 1989]. The HCV RNA in the serum and CSF samples was quantified using the branched-DNA (b-DNA) method (Quantiplex-HCV-bDNA, Chiron Corp., Emeryville, CA) [Urdea et al., 1991], and quantitative PCR (Amplicor-HCV-Monitor, Roche Diagnostics, Basel, Switzerland) [Young et al., 1993]. The same procedures were used for both the CSF and the serum samples. HCV genotypes were classified using a line probe assay (LiPa, Innogenetics, Haven, Belgium) [Stuyver et al., 1993]. All samples were tested at least twice, under blinded conditions. Anti-HCV antibodies were determined in serum and CSF samples by a second-generation enzyme-linked immunosorbent assay ELISA (Ortho Diagnostic Systems, Raritan, NJ).

RESULTS

HCV-RNA Detection

HCV-RNA was detected in the serum of 19/21 patients (90.4%) and in the CSF of 5/21 (23.8%); there was complete concordance for the presence of HCV-RNA in CSF and serum. Table I shows the physicochemical properties of the CSF of these five patients. Two of these patients had no radiological evidence of diffuse or focal brain lesions, absence of *Cryptococcus* antigen in the CSF, and no signs of HIV-related encephalopathy; the CSF diagnostic procedures remained inconclusive in both patients.

HCV-RNA Quantification e Viral Genotype

HCV genotype was characterized in the serum and CSF samples of the HCV-RNA-positive patients in order to determine whether any particular genotype may have neurotropism: genotype 1a was found in one patient, 1b in two, 3a in one, and 4a in one; the same genotype was also found in the corresponding sera. Two different methods with different sensitivities were used for HCV quantitation, in order to measure both high and low viral titers more accurately. The mean number of HCV-RNA copies measured by quantitative PCR in serum was 1.02×10^6/ml in CSF-negative patients, and 1.67×10^6/ml in CSF-positive patients ($P = 0.5$, Student's t-test). The mean number of serum RNA copy equivalents, measured by means of b-DNA was 9.06×10^6 Eq/ml in CSF-negative cases and 14.95×10^6/ml in CSF-positive cases ($P = 0.3$, Student's t-test). The viremia level measured by b-DNA was higher than that measured by quantitative PCR. This result can be explained by the fact that the b-DNA assay amplifies the signal, rather than the genomic target sequence; consequently, the plateau effect that occurs during PCR amplification with high template concentration [Wang et al., 1989] is not reached. However, as quantitative PCR is more sensitive than b-DNA for detecting smaller amounts of virus particles, it was reasonable to use both methods. The amount of HCV-RNA in the CSF samples found to be positive by nested PCR was less than the cutoff point of 3.5×10^5 Eq/ml in 5/5 samples measured by b-DNA, and in 3/4 samples measured by quantitative PCR (cutoff value: $<10^3$ copies/ml); in one CSF sample, 4.2×10^3 copies/ml were measured by quantitative PCR. The virological and biochemical findings in the CSF-positive patients are summarized in Table I.

Follow-up Samples

Replicate CSF and serum samples were available for seven patients. HCV-RNA was not found in four patients, and the two patients with HCV-RNA in the CSF at first determination continued to be positive at the second. In the case of the last patient, seven CSF samples were tested over a period of 20 months, three of which (nonconsecutive) proved to be HCV-RNA positive; the corresponding serum samples of this patient were all HCV-RNA positive.

Controls of CSF Contamination

In order to exclude possible contamination of the CSF supernatants by mononuclear cells that may have been harboring HCV, DNA-PCR for an HLA-DQ-α locus was carried out in the HCV-RNA-positive samples and found to be negative. Red blood cells were never detected by microscopical examination of the CSF, ruling out possible contamination of the CSF sample by blood. Anti-HCV was not found in any of the CSF samples despite the strong reactivity of the corresponding sera (optical density >2.0). Finally, the finding of HCV-RNA in the CSF was not influenced by CNS infection with other viruses or acquired immunodeficiency syndrome (AIDS)-related pathogens.

DISCUSSION

This study showed the presence of HCV in the CSF of a significant number (5/21) of patients with HIV/HCV co-infection, with the PCR results generally confirmed in the replicate CSF samples. The first hypothesis to explain these findings is that the CSF samples were contaminated by blood at the time of the lumbar puncture, but this is unlikely because of the absence of blood in the CSF samples and, above all, because of the reproducibility of the PCR results in the follow-up samples of seven patients. Thus, HCV-RNA was not found in subsequent CSF samples from 4 patients, and it was found in the replicate CSF samples of three other patients. These results exclude the likelihood of CSF contamination with blood in the study samples.

The second possibility is that the detection of HCV in CSF may mirror passive transfer of virions in highly viremic patients, probably through the brain capillary and the choroidal plexus, or even through the peripheral nerves; however, the patients with undetectable HCV-RNA in the CSF also had high viremia levels.

The third hypothesis is that the presence of HCV genomic sequences in a significant number of CSF samples, as reported in this study, was related to a productive infection inside the CNS. Intrathecal synthesis of anti-HCV antibodies, which may be considered to argue against the hypothesis of replicative HCV infection inside the CNS, was not found. However, it was previously shown that viral infection of the brain may also occur in the absence of the intrathecal synthesis of specific antibodies [Scheld et al., 1991]. Furthermore, it is stressed that all the patients had advanced HIV infection and that immunodeficiency may have increased the likelihood of CSF/CNS HCV infection. However, this hypothesis needs further study to demonstrate HCV replication in the CNS.

In conclusion, the results suggest that the detection of HCV genomic sequences in CSF may be related to passive transfer or the active production of HCV virions inside the CNS. Both hypotheses raise the possibility that the CNS may act as an extrahepatic site for HCV replication. In addition to its possible neurological implications, this finding could be of primary importance for the treatment of chronic hepatitis C given that the antiviral effects of interferon in the CNS are probably limited. This is the first report showing the presence of HCV in the CSF. Further studies are in progress to test for the presence of HCV genomic sequences in the CSF of HIV-negative patients with undefined neurological disorders.

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